Susceptibility of the Lesser Peachtree Borer (Lepidoptera: Sesiidae) to Entomopathogenic Nematodes Under Laboratory Conditions

DAVID I. SHAPIRO-ILAN[1] AND TED E. COTTRELL

USDA–ARS, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA 31008

Environ. Entomol. 35(2): 358–365 (2006)

ABSTRACT The lesser peachtree borer, *Synanthedon pictipes* (Grote and Robinson), is an important pest of *Prunus* spp. We determined the susceptibility of *S. pictipes* to six entomopathogenic nematode species: *Heterorhabditis bacteriophora* Poinar, *H. indica* Poinar, Karunakar and David, *H. marelatus* Liu and Berry, *Steinernema carpocapsae* (Weiser), *S. feltiae* (Filipjev), and *S. riobrave* Cabanillas, Poinar and Raulston. Nematode virulence in *S. pictipes* was compared with virulence in two known susceptible hosts, *Galleria mellonella* L. and *Tenebrio molitor* L. In *S. pictipes*, the steinernematids were more virulent than the heterorhabditids, the virulence of *S. carpocapsae* was greater than *S. riobrave*, with *S. feltiae* being intermediate between the two, and no differences in virulence were detected among the heterorhabditids. Each nematode exhibited similar or greater virulence to *S. pictipes* than to *T. molitor*, and the steinernematids' virulence to *S. pictipes* was greater or similar to *H. bacteriophora* or *H. marelatus* virulence in *G. mellonella*. A quadratic dose–response relationship was detected between *S. carpocapsae* and *S. pictipes*, and an LC_{50} was estimated to be 7.99. Comparisons of steinernematid reproductive potential per host, or per milligram host, generally indicated the highest production in *G.* mellonella; production in *S. pictipes* was similar or greater than in *T. molitor*. In *S. pictipes*, no differences in reproduction were detected among nematode species. Based on our findings and other studies on related insect species, we conclude that the prospects for controlling *S. pictipes* with entomopathogenic nematodes are promising (particularly with *S. carpocapsae* and *S. feltiae*), and field testing is warranted.

KEY WORDS biological control, entomopathogenic nematode, *Heterorhabditis*, *Steinernema*, *Synanthedon pictipes*

THE LESSER PEACHTREE BORER, *Synanthedon pictipes* (Grote and Robinson), is an important pest of peach, *Prunus persica* L., and other *Prunus* spp. in the eastern United States (Johnson et al. 2005). Generally, two generations of *S. pictipes* occur per year. Depending on climactic conditions, adult moth emergence can occur in January and February, but more typically, first brood emergence begins in March and peaks in April and May, and the second brood's emergence peaks between July and September. Adult moths lay eggs on the trunk and limbs usually in cracks in the tree's bark and often in the crotch or near injured areas (Bobb 1959, Johnson et al. 2005). Larvae tunnel into the inner bark and cambium where they feed and develop; second-generation larvae overwinter in the tunnels. Damage from larval feeding reduces tree vigor and in high infestations can lead to loss of tree limbs or render the entire tree unsalvageable (Johnson et al. 2005).

Current control recommendations depend on the use of chemical insecticides (Johnson et al. 2005). In the southeastern United States, the sole recommendation for *S. pictipes* control consists of handgun application of chemical insecticides (primarily the organophosphate chlorpyrifos) to the scaffold limbs, but efficacy is only marginal (Brannen et al. 2005). Because of environmental and regulatory concerns associated with such chemical use (Luckman and Metcalf 1982, National Research Council 1989, Hamilton et al. 1997, Cohen 2000), development of alternative strategies is warranted. To date, only a few alternative control strategies for *S. pictipes* control have been studied, such as the natural product abamectin, which exhibited poor efficacy (Yonce and Taylor 1992), and mating disruption (Snow et al. 1985, Pfeiffer et al. 1991). Application of biological control agents has not been explored as an option. Entomopathogenic nematodes may have potential as a biocontrol alternative for *S. pictipes* suppression.

Entomopathogenic nematodes (families Steinernematidae and Heterorhabditidae) are obligate parasites of insects (Poinar 1990, Adams and Nguyen 2002). These nematodes are mutualistically associated with bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp. for steinernematids and heterorhabditids, respectively). Infective juveniles (IJs), the only free-living

[1] Corresponding author, e-mail: dshapiro@saa.ars.usda.gov.

stage, enter hosts through natural openings (mouth, anus, and spiracles), or in some cases, through the cuticle. After entering the host's hemocoel, nematodes release their symbiotic bacteria, which are primarily responsible for killing the host, defending against secondary invaders, and providing the nematodes with nutrition (Dowds and Peters 2002). The nematodes molt and complete up to three generations within the host after which IJs exit the cadaver to search out new hosts (Kaya and Gaugler 1993).

Entomopathogenic nematodes are effective biocontrol agents of a variety of economically important insect pests (Klein 1990, Shapiro-Ilan et al. 2002a, Grewal et al. 2005). A number of studies indicate aboveground applications of entomopathogenic nematodes can result in high levels of control for a variety of sesiid pests including several *Synanthedon* spp. (Miller and Bedding 1982, Deseö and Miller 1985, Kaya and Brown 1986, Begley 1990, Nachtigall and Dickler 1992). However, virulence or efficacy levels against sesiid pests can vary among different hosts and nematode species or strains (Bedding and Miller 1981, Deseö and Miller 1985, Saunders and All 1985, Kaya and Brown 1986, Cossentine et al. 1990, Nachtigall and Dickler 1992, Smith-Fiola et al. 1996, Williams et al. 2002).

Our overall goal is to determine the potential to use entomopathogenic nematodes for *S. pictipes* suppression. The susceptibility of *S. pictipes* to entomopathogenic nematodes has not been previously reported. A critical component for success in any biocontrol program with entomopathogenic nematodes is matching the most suitable nematode with the target host, and relative virulence among different nematodes is one of the important factors to consider in determining suitability (Georgis and Gaugler 1991, Shapiro-Ilan et al. 2002a). Thus, in this study, our primary objective was to estimate the susceptibility of *S. pictipes* to several entomopathogenic nematodes. We measured the virulence of six commercially available nematode species to *S. pictipes* larvae. Furthermore, to estimate relative susceptibility, we compared nematode virulence in *S. pictipes* to virulence in larvae of the greater wax moth, *Galleria mellonella* L., and yellow mealworm, *Tenebrio molitor* L., which are two hosts that are known to be susceptible to entomopathogenic nematodes and are used in in vivo nematode production (Shapiro-Ilan and Gaugler 2002). In addition to virulence, the ability of a nematode to recycle in the target host may also contribute to successful biocontrol (Smits 1996, Shields et al. 1999). Thus, we compared the reproductive potential of three nematode species (the ones found to be most virulent to *S. pictipes*) in *S. pictipes*, *G. mellonella*, and *T. molitor*.

Materials and Methods

Insects, Nematodes, and Experimental Conditions. Before experimentation, all nematodes were cultured in parallel at 25°C in *G. mellonella* according to procedures described by Kaya and Stock (1997). After harvest, IJs were stored at 13°C for <3 wk before they were used in experiments. *S. pictipes* larvae (approximately fifth and sixth instar; 0.04 ± 0.02 g) were collected from peach orchards near Byron, GA, and used in bioassays the following day. The larvae were manually extracted from infested trees (Bobb 1959) using flat screwdrivers to pry into tunnels and soft forceps to remove larvae. For all experiments described below, larval extraction was conducted between 15 February and 4 March 2005, except in the second reproduction trial, when larvae were collected 22 September 2005. Late-instar *T. molitor* (0.06 ± 0.01 g) and last-instar *G. mellonella* (0.23 ± 0.06 g) were obtained from Southeastern Insectaries (Perry, GA) and Webster's Waxie Ranch (Webster, WI), respectively. All experiments were conducted in the laboratory at 25°C.

Virulence Assays. Virulence to *G. mellonella*, *S. pictipes*, and *T. molitor* was compared among the following entomopathogenic nematodes: *Heterorhabditis bacteriophora* Poinar (VS strain), *H. indica* Poinar, Karunakar and David (HOM1 strain), *H. marelatus* Liu and Berry (Point Reyes strain), *Steinernema carpocapsae* (Weiser) (all strains), *S. feltiae* (Filipjev) (SN strain), and *S. riobrave* Cabanillas, Poinar and Raulston (355 strain). The species tested comprise representatives of both entomopathogenic nematode genera and both types of foraging strategy (ambusher and cruiser; Lewis 2002). The assay arena consisted of an inverted 60-mm-diameter petri dish with a single filter paper (Whatman No. 1) lining the bottom (lid). Approximately 20 IJs in 0.35 ml tap water were pipetted onto the filter paper of each dish. The rate of application was chosen based on preliminary trials conducted to estimate a distinguishing dose (unpublished data). Control dishes received an equal amount of tap water (no nematodes). A single insect was added to each dish immediately after nematode inoculation. Insect mortality was assessed 48 h after inoculation. The experiment was set up as a factorial (with nematode and host as the main effects) in a randomized block design. Each treatment contained four replicates of seven dishes, and the entire experiment was repeated once (as two trials).

In addition to the virulence comparisons among nematodes, we studied the dose–response relationship between *S. pictipes* and the nematode that produced the (numerically) highest level of mortality, i.e., *S. carpocapsae* (see Results section). Application rates included 0, 10, 20, 30, 40, and 50 IJs per insect. Assay conditions and mortality assessment (48 h) were as described above. There were 20 insects per concentration, except for the 10 IJ concentration where only 19 insects were used because 1 insect was accidentally crushed before mortality assessment.

Reproduction Assays. Nematode reproductive potential was compared in *G. mellonella*, *S. pictipes*, and *T. molitor*. The reproduction assays were limited to *S. carpocapsae*, *S. feltiae*, and *S. riobrave* because these nematodes caused greater mortality in *S. pictipes* than the other three nematodes in the virulence assays (see Results section). To assess reproduction, individual

insect cadavers from the second trial of the virulence assay were each placed on White traps (Kaya and Stock 1997). The number of emerging IJs was determined through dilution counts 21 d after inoculation when emergence had essentially ceased. Our initial goal was to include 10 replicate cadavers for each nematode–host combination. This goal was reached in all treatments except for *S. riobrave* in *G. mellonella* (which only contained nine replicates) and *S. feltiae* in *T. molitor* (which only contained four replicates). Thus, we conducted a second trial in which all three nematodes species were again exposed to the three insect hosts as described above. A total of 16 insects of each host species were exposed to nematodes from which 9 infected insects of each were placed on White traps. The final number of infected insects in the combined reproduction trials was 19 for all treatments except *S. feltiae* in *T. molitor*, which had 14 replicates (i.e., insects). The trials were set up in a completely randomized design. Because reproductive yield can vary based on the host's mass (Shapiro-Ilan and Gaugler 2002), we measured the mass of each insect cadaver and determined the number of IJs produced on a per insect as well as a per milligram of insect basis.

Data Analysis. Because of a lack of independence, the main treatment effects in the factorial experiment addressing virulence were analyzed separately through analysis of variance (ANOVA) (Cochran and Cox 1957, SAS Institute 2001). The nematode effect was analyzed within each host by comparing mortality observed in the six nematode treatments to each other and in the control. The host effect was analyzed for each nematode; in this case, however, to avoid potential bias caused by unequal control mortality, Abbott's formula (Abbott 1925) was applied to the data before analysis. Probit analysis was used to determine the LC_{50}, LC_{75}, and LC_{90} for *S. carpocapsae* in *S. pictipes*, and linear and quadratic regression was applied to the data to further elucidate the dose–response relationship (SAS Institute 2001). Percentage data in the virulence assays comparing nematode treatments were arcsine transformed before analysis, and numerical data in the reproduction assays (number of IJs produced) were square root transformed before ANOVA (Steel and Torrie 1980, SAS Institute 2001). The Student-Newman-Keuls' test was used to elucidate treatment effects when a significant F value ($P \leq 0.05$) was detected in ANOVA (SAS Institute 2001).

Results

Virulence Assays. An interaction between main effects (nematode × host) was detected ($F = 4.68$; df = 12,147; $P < 0.0001$). No interaction was detected between trial and nematode treatment effects ($F = 1.12$; df = 6,152; $P = 0.35$) or trial and host effects ($F = 2.72$; df = 2,133; $P = 0.69$). Therefore, data from the two trials were combined.

Virulence varied among nematode species within each host. In *S. pictipes*, the virulence of steinernematids was greater than that of the heterorhabditids, *S. carpocapsae* was more virulent than *S. riobrave*, with *S. feltiae* being intermediate, and there were no differences detected in heterorhabditid virulence; mortality in all nematode treatments was higher than in the controls ($F = 20.71$; df = 6,55; $P < 0.0001$; Fig. 1). In *G. mellonella*, *H. indica*, *S. carpocapsae*, *S. feltiae*, and *S. riobrave* caused greater mortality than *H. marelatus*, which caused greater mortality than *H. bacteriophora*, and mortality in all nematode treatments was higher than in the control ($F = 44.11$; df = 6,55; $P < 0.0001$; Fig. 1). In *T. molitor*, *H. indica*, *S. carpocapsae*, *S. feltiae*, and *S. riobrave* caused greater mortality than *H. marelatus*, which caused greater mortality than *H. bacteriophora*, and except for *H. bacteriophora*, mortality in all nematode treatments was higher than in the control ($F = 27.83$; df = 6,55; $P < 0.0001$; Fig. 1).

Differential susceptibility to host insects was detected within each nematode species ($F = 13.01, 44.65, 12.56, 22.79, 22.01$, and 32.89 for *H. bacteriophora*, *H. indica*, *H. marelatus*, *S. carpocapsae*, *S. feltiae*, and *S. riobrave*, respectively; df = 2,20; $P < 0.0004$ for all species; Fig. 2). Three of the nematode species (*H. bacteriophora*, *S. carpocapsae*, and *S. feltiae*) exhibited greater virulence to *S. pictipes* than to *T. molitor*, and no difference in host susceptibility between the two insects was detected in the other three nematode species (Fig. 2). Each nematode caused greater mortality in *G. mellonella* than in *S. pictipes* or *T. molitor* except *H. bacteriophora*, for which no difference in mortality was detected between *G. mellonella* and *S. pictipes* (Fig. 2). Additionally, when considering relative host susceptibility, we noted that the mortality of *S. pictipes* caused by the steinernematids (*S. carpocapsae*, 80.4 ± 8.5; *S. feltiae*, 67.9 ± 8.0; *S. riobrave*, 51.8 ± 9.3) was clearly equal or greater than mortality of *G. mellonella* caused by *H. bacteriophora* (30.4 ± 7.5) or *H. marelatus* (49.0 ± 4.8).

Regression analysis indicated a quadratic relationship between *S. carpocapsae* concentration and *S. pictipes* mortality ($R^2 = -0.95$, $y = -0.05x^2 + 4.5x + 6.6$; [SEs for parameter estimates are 0.02, 0.8, and 8.9 for x^2, x, and the intercept, respectively] $P = 0.01$; error mean square = 97.4; Fig. 3). Probit analysis revealed an LC_{50} of 7.99 (95% CL: 0.91–13.44), LC_{75} of 19.3 (95% CL: 9.78–29.50), and LC_{90} of 42.85 (95% CL: 28.40–176.15; $n = 20$, slope = 1.76 ± 0.60, $\chi^2 = 1.1$).

Reproduction Assays. When considering yield per insect or yield per milligram insect, an interaction between main effects was detected ($F = 4.93$; df = 4,152; $P = 0.0009$ for the per insect variable, and $F = 3.74$; df = 4,151; $P = 0.006$ for per milligram); thus, treatment combinations (nematode × host) were analyzed individually. No interaction was detected between trial and treatment effects ($F = 1.1$; df = 8,148; $P = 0.37$ for per host and $F = 1.34$; df = 8,147; $P = 0.23$ for per milligram). Therefore, data from the two trials were combined. Treatment differences in reproductive potential were detected for yield per insect ($F = 38.97$; df = 8,148; $P < 0.0001$) and yield per milligram insect ($F = 14.17$; df = 8,147; $P < 0.0001$). When yield per insect was compared, reproduction was greatest in *G. mellonella* for all nematode species, with no

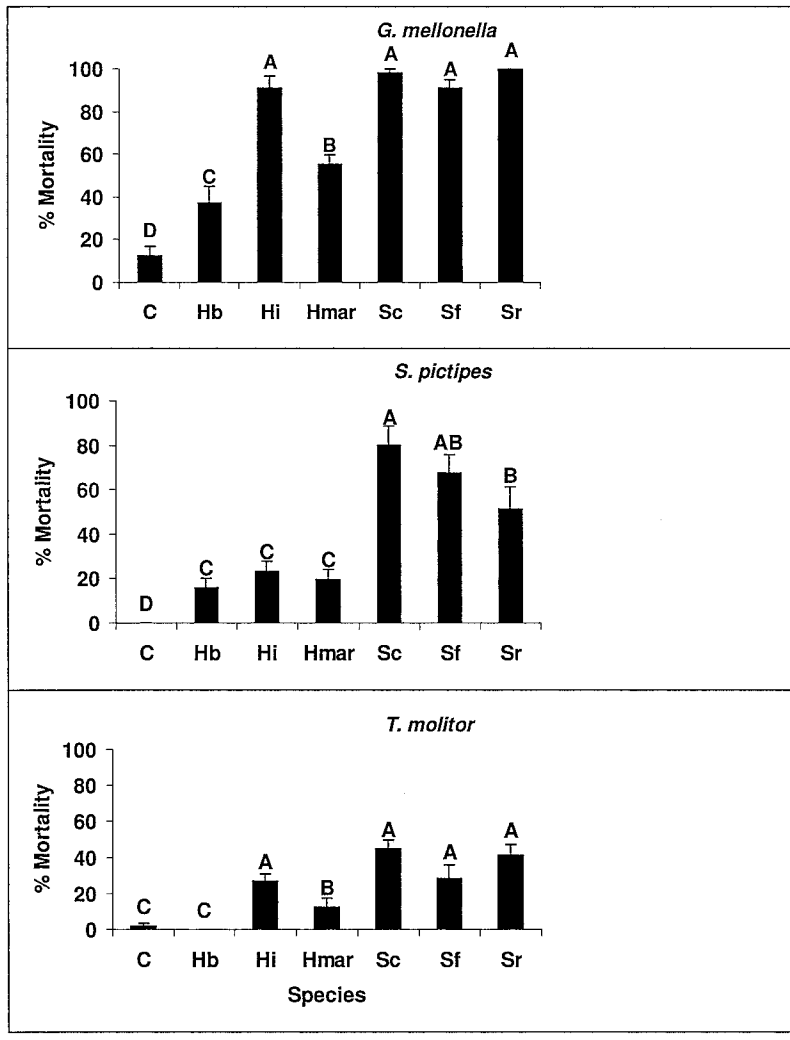

Fig. 1. Mean ± SEM percentage mortality of *G. mellonella*, *S. pictipes*, and *T. molitor* 48 h after exposure to entomopathogenic nematodes or an untreated control under laboratory conditions. Hb, *H. bacteriophora* (VS strain); Hi, *H. indica* (HOM1 strain); Hmar, *H. marelatus* (Point Reyes strain); Sc, *Steinernema carpocapsae* (All Strain); Sf, *S. feltiae* (SN strain); Sr, *S. riobrave* (355 strain); C, control. Different letters above bars indicate statistical significance (Student-Newman-Keuls test, $P \leq 0.05$).

differences between the other two hosts except that *S. carpocapsae* reproduction in *S. pictipes* was greater than in *T. molitor* (Fig. 4). When yield per milligram insect was compared, the lowest reproduction was observed in *T. molitor* for all nematode species and was highest in *G. mellonella* for all nematodes except for *S. feltiae* (where reproduction in *G. mellonella* and *S. pictipes* were not different; Fig. 4). Reproduction in *S. pictipes* did not differ among nematode species on a per host or per milligram basis (Fig. 4).

Discussion

Although all six entomopathogenic nematode species tested were found to be pathogenic to *S. pictipes*, virulence among them varied considerably. Our results are consistent with several other studies that indicated superior virulence in steinernematids compared with heterorhabditids when measuring susceptibility of *Synanthedon* spp. (Deseö and Miller 1985, Nachtigall and Dickler 1992). For example, Nachtigall and Dickler (1992) reported a 75% reduction of *Synanthedon myopaeformis* (Borkhausen) after field applications of *S. feltiae*, yet *Heterorhabditis* sp. had no effect. Additionally, Bedding and Miller (1981) observed greater virulence in *S. feltiae* compared with *H. bacteriophora* when applied for suppression of the currant borer, *Synanthedon tipuliformis* (Clerck), but unlike our results, these authors observed lower virulence in *S. carpocapsae* compared with *S. feltiae* (we did not detect a difference between the two). Also, in contrast with our results, when measuring ento-

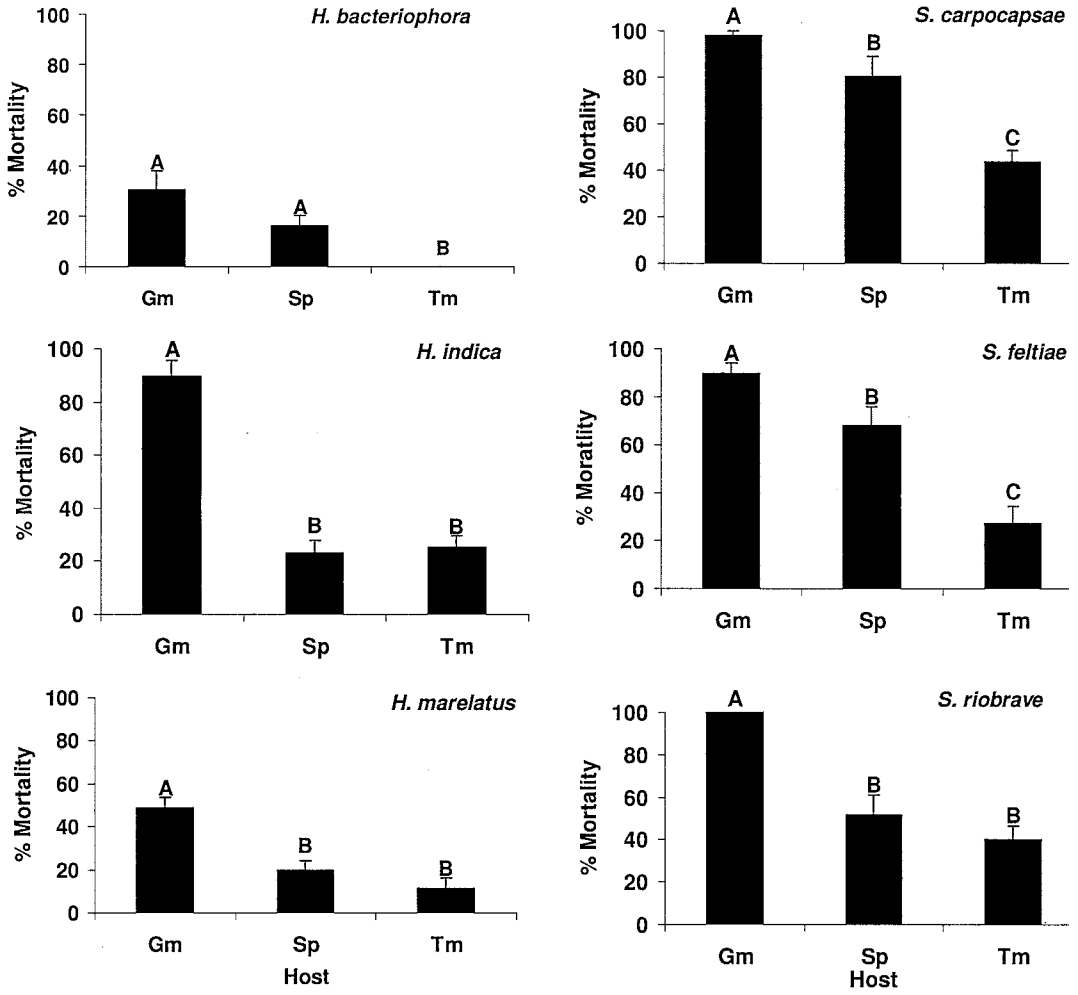

Fig. 2. Mean ± SEM percentage control of *G. mellonella*, *S. pictipes*, and *T. molitor* after 48 h of exposure to entomopathogenic nematodes, *Heterorhabditis* spp., and *Steinernema* spp. under laboratory conditions. Control mortality was corrected through the formula of Abbott (1925). Different letters above bars indicate statistical significance (Student-Newman-Keuls test, $P \leq 0.05$).

mopathogenic nematode laboratory virulence to another sessiid, the grape root borer, *Vitacea polistiformis* (Harris), Williams et al. (2002) reported higher virulence in *H. bacteriophora* (GPS11 strain) and *H. zealandica* Poinar than in *S. carpocapsae*, *S. glaseri*, and *S. riobrave*, yet a number of other *H. bacteriophora* strains exhibited substantially lower virulence. Similar to our study, Williams et al. (2002) observed higher virulence in *S. carpocapsae* than in *S. riobrave*. We also observed higher virulence of *S. carpocapsae* than *S. riobrave* in the peachtree borer, *Synanthedon exitiosa* (Say) in a laboratory and field trial (unpublished data). The results of these studies confirm that entomopathogenic nematode virulence or efficacy in control of sesiid pests varies with the particular nematode and host as well as among different nematode strains and environmental conditions.

Our data indicate that *S. pictipes* is also a relatively susceptible host. The virulence of each nematode species to *S. pictipes* was equal or greater than virulence to *T. molitor*, and the virulence of the steinernematids was equal or greater than the virulence of *H. bacteriophora* or *H. marelatus* to *G. mellonella*. Using virulence in *G. mellonella* and *T. molitor* as an indicator of relative susceptibility (of *S. pictipes*) is reasonable because these two insects are considered to be susceptible hosts and are thus widely used in laboratory rearing and are suitable to mass in vivo culture of a variety of nematode species (Blinova and Ivanova 1987, Woodring and Kaya 1988, Shapiro-Ilan et al. 2002b). Indeed all of the nematode species we tested were reared commercially in either *G. mellonella* or *T. molitor* or both (D.I.S., unpublished data). Furthermore, the broad innate susceptibility of *G. mellonella* is evidenced by the insect's common use as a bait insect to isolate wild entomopathogenic nematode strains (Kaya and Stock 1997, Hominick 2002). It is conceivable that, during routine laboratory culturing

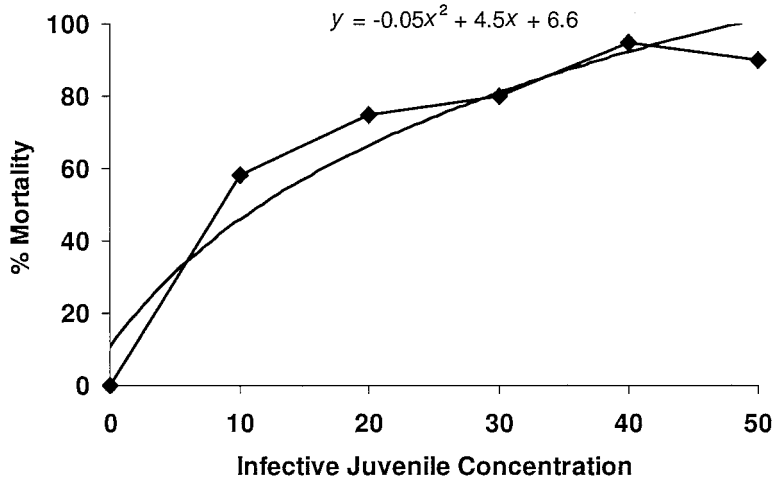

Fig. 3. Effect of *S. carpocapsae* IJ concentration on *S. pictipes* mortality under laboratory conditions. Line without dots represents a predicted curve based on linear and quadratic responses.

before our experiments, nematode virulence to *G. mellonella* may have increased because of inadvertent selection (Stuart and Gaugler 1996). If so, the observed nematode virulence to *S. pictipes* compared with virulence to *G. mellonella* is even more encouraging.

Because of differences in nematode strains and experimental conditions, it is difficult to compare virulence from one study to another. Nonetheless, additional evidence to support our conclusion of relatively high host susceptibility in *S. pictipes* stems from a comparison of the LC_{50} we obtained for *S. carpocapsae* in *S. pictipes*, with the LC_{50} calculated for other nematodes combinations that were considered to be highly virulent. For example, the LC_{50} we obtained is similar (95% CL does not overlap) to the LC_{50} calculated for *S. carpocapsae* in the pickleworm, *Diaphania nitidalis* (Stoll) (Shannag et al. 1994), *Steinernema scarabaei* (Stock and Koppenhöfer) in the European chafer, *Rhizotrogus majalis* (Razoumowsky) or Japanese beetle, *Popillia japonica* Newman (Cappaert and Koppenhöfer 2003), and *H. bacteriophora* in the clover root curculio, *Sitona hispidulus* (Fabricius) (Loya and Hower 2003).

The reproduction assays also indicated host susceptibility in *S. pictipes.* All three nematodes tested were capable of reproducing in *S. pictipes.* The ability to reproduce in the target host may result in recycling and thus additional pest suppression (Smits 1996, Shields et al. 1999); we hypothesize that nematode reproduction inside *S. pictipes* tunnels is likely because entomopathogenic nematodes have been observed to reproduce within tunnels of other *Synanthedon* spp. (Miller and Bedding 1982). Differences in insect mass

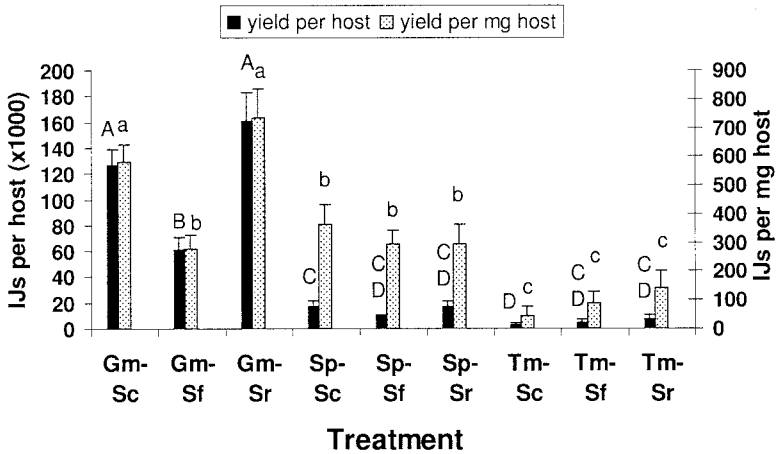

Fig. 4. Mean ± SEM number of entomopathogenic nematodes (Sc, *S. carpocapsae;* Sf, *S. feltiae*; Sr, *S. riobrave*) produced in insect hosts (Gm, *G. mellonella*; Sp, *S. pictipes*; Tm, *T. molitor*). IJs, nematode infective juveniles. Different capital and lowercase letters above bars indicate statistical differences for IJs per host and IJs/mg/insect, respectively (Student-Newman-Keuls test, $P \leq 0.05$).

can be an important consideration when comparing reproduction among nematodes (Flanders et al. 1996, Shapiro-Ilan et al. 2002b). Our results, however, did not indicate substantial differences in the analysis of total reproduction per insect relative to the weight-based analysis, although there was more substantial separation between yields in *S. pictipes* versus *T. molitor* in the latter analysis. Overall, our data indicate that steinernematid reproduction in *S. pictipes* is more efficient than in *T. molitor* but less than in *G. mellonella.*

Our laboratory data support the premise that entomopathogenic nematodes might fit into an *S. pictipes* management strategy. An alternative strategy is needed because the current recommendation of chemical sprays to the affected areas are only poorly to moderately effective (Brannen et al. 2005). In contrast, high levels of efficacy (ranging from 74 to 94% mortality) have been observed from aboveground spray applications of entomopathogenic nematodes for control of *Synanthedon* spp. in various commodities such as apples, *Malus* spp. (Deseö and Miller 1985, Nachtigall and Dickler, 1992), alder, *Alnus* spp. (Kaya and Brown 1986), and black currants, *Ribes nigrum* L. (Miller and Bedding 1982). To a large extent, the poor efficacy in chemical applications is likely caused by insufficient penetration into the larval tunnels. Entomopathogenic nematodes have been observed to move within *S. tipuliformis* tunnels (Miller and Bedding 1982). We hypothesize that, similar to the examples in other systems (Miller and Bedding 1982, Deseö and Miller 1985, Kaya and Brown 1986, Nachtigall and Dickler, 1992), high levels of efficacy can also be achieved through application of entomopathogenic nematodes (particularly steinernematids such as *S. carpocapsae* and *S. feltiae*) to *S. pictipes*–infested trunks and limbs. However, observations of virulence in the laboratory do not necessarily predict effective pest control under field conditions; a variety of traits in the nematodes and other biotic and abiotic factors contribute to nematode field efficacy (Shapiro-Ilan et al. 2002a). Field experiments are planned to test our hypothesis.

Acknowledgments

We thank A. Amis, R. Auman, W. Evans, K. Halat, R. Laster, G. Lathrop, and W. Turner for technical assistance and K. Nguyen, P. Stock, Certis USA (now Becker Underwood), and Integrated Biocontrol Systems for providing nematode cultures.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Adams, B. J., and K. B. Nguyen. 2002. Taxonomy and systematics, pp. 1–34. *In* R. Gaugler (ed.), Entomopathogenic nematology. CABI, New York.

Bedding, R. A., and L. A. Miller. 1981. Disinfecting blackcurrant cuttings of *Synanthedon tipuliformis,* using the insect parasitic nematode *Neoaplectana bibionis.* Environ. Entomol. 10: 449–453.

Begley, J. W. 1990. Efficacy against insects in habitats other than soil, pp. 215–232. *In* R. Gaugler and H. K. Kaya (eds.), Entomopathogenic nematodes in biological control. CRC, Boca Raton, FL.

Blinova S. L., and E. S. Ivanova. 1987. Culturing the nematode-bacterial complex of *Neoaplectana carpocapsae* in insects, pp. 13–21. *In* M. D. Sonin (ed.), Helminths of insects. American Publishing, New Delhi, India.

Bobb, M. L. 1959. The biology of the lesser peach tree borer in Virginia. J. Econ. Entomol. 52: 634–636.

Brannen, P., D. Horton, B. Bellinger, and D. Ritchie. 2005. 2005 Southeastern peach, nectarine, and plum pest management and culture guide. University of Georgia Cooperative Extension Service, Athens, GA.

Cappaert, D. L., and A. M. Koppenhöfer. 2003. *Steinernema scarabaei*, an entomopathogenic nematode for control of the European chafer. Biol. Control 28: 379–386.

Cochran, W. G., and G. M. Cox. 1957. Experimental designs. Wiley, New York.

Cohen, S. 2000. Recent examples of pesticide assessment and regulation under FQPA. Ground Water Monit. Remed. 20: 41–43.

Cossentine, J. E., F. L. Banham, and L. B. Jensen. 1990. Efficacy of the nematode *Heterorhabditis heliothidis* (Rhabditida: Heterorhabditidae) against peachtree borer, *Synanthedon exitiosa* (Lepidoptera: Sesiidae) in peach trees. J. Entomol. Soc. Br. Columbia. 87: 82–84.

Deseö, K. V., and L. A. Miller. 1985. Efficacy of entomogenous nematodes, *Steinernema* spp., against clearwing moths, *Synanthedon* spp., in north Italian apple orchards. Nematologica 31: 100–108.

Dowds, B.C.A., and A. Peters. 2002. Virulence mechanisms, pp. 79–98. *In* R. Gaugler (ed.), Entomopathogenic nematology. CABI, New York.

Flanders K. L., J. M. Miller and E. J. Shields. 1996. In vivo production of *Heterorhabditis bacteriophora* 'Oswego' (Rhabditida: Heterorhabditidae), a potential biological control agent for soil inhabiting insects in temperate regions. J. Econ. Entomol. 89: 373–380.

Georgis, R., and R. Gaugler. 1991. Predictability in biological control using entomopathogenic nematodes. J. Econ. Entomol. 84: 713–720.

Grewal, P. S., R.-U. Ehlers, and D. I. Shapiro-Ilan, (eds.). 2005. Nematodes as biocontrol agents. CABI, New York.

Hamilton, G. C., M. G. Robson, G. M. Ghidiu, R. Samulis, and E. Prostko. 1997. 75% adoption of integrated pest management by 2000? A case study from New Jersey. Am. Entomol. 43: 74–78.

Hominick, W. M. 2002. Biogeography, pp. 115–145. *In* R. Gaugler (ed.), Entomopathogenic nematology. CABI, New York.

Johnson, D., T. Cottrell, and D. Horton. 2005. Lesser peachtree borer, pp. 270–272. *In* D. Horton and D. Johnson (eds.), Southeastern peach growers handbook. University of Georgia Cooperative Extension Service, Athens, GA.

Kaya, H. K., and L. R. Brown. 1986. Field application of entomogenous nematodes for biological control of clearwing moth borers in alder and sycamore trees. J. Arboric. 12: 150–154.

Kaya, H. K., and R. Gaugler. 1993. Entomopathogenic nematodes. Annu. Rev. Entomol. 38: 181–206.

Kaya, H. K., and S. P. Stock. 1997. Techniques in insect nematology, pp. 281–324. *In* L.A. Lacey (ed.), Manual of techniques in insect pathology. Academic, San Diego, CA.

Klein, M. G. 1990. Efficacy against soil-inhabiting insect pests, pp. 195–214. *In* R. Gaugler and H. K. Kaya (eds.),

Entomopathogenic nematodes in biological control. CRC, Boca Raton, FL.

Lewis, E. E. 2002. Behavioral ecology, pp. 205–224. *In* R. Gaugler (ed.), Entomopathogenic nematology. CABI, New York.

Loya, L. J., and A. A. Hower, Jr. 2003. Infectivity and reproductive potential of the Oswego strain of *Heterorhabditis bacteriophora* associated with life stages of the clover root curculio, *Sitona hispidulus.* J. Invertebr. Pathol. 83: 63–72.

Luckman, W. H., and R. L. Metcalf. 1982. The pest-management concept, pp. 1–32. *In* R. L. Metcalf and W. H. Luckman (eds.), Introduction to insect pest management. Wiley, New York.

Miller, L. A., and R. A. Bedding. 1982. Field testing of the insect parasitic nematode *Neoaplectana bibionis* [Nematoda: Steinernematidae] against currant borer moth, *Synanthedon tipuliformis* [Lep: Sesiidae] in blackcurrants. Entomophaga 27: 109–114.

Nachtigall, G., and E. Dickler. 1992. Experiences with field applications of entomogenous nematodes for biological control of cryptic living insects in orchards. Acta Phytopath. Entomol. Hung. 27: 485–490.

National Research Council. 1989. Alternative agriculture. National Academy Press, Washington, DC.

Pfeiffer, D. G., J. C. Killian, E. G. Rajotte, L. A. Hull, and J. W. Snow. 1991. Mating disruption by lesser peachtree borer (Lepidoptera: Sesiidae) in Virginia and Pennsylvania peach orchards. J. Econ. Entomol. 84: 218–223.

Poinar, G. O., Jr. 1990. Biology and taxonomy of Steinernematidae and Heterorhabditidae, pp. 23–62. *In* R. Gaugler and H. K. Kaya (eds.), Entomopathogenic nematodes in biological control. CRC, Boca Raton, FL.

SAS. 2001. SAS software: version 8.2. SAS Institute, Cary, NC.

Saunders, M., and J. N. All. 1985. Association of entomophilic rhabditoid nematode populations with natural control of first-instar larvae of the grape root borer, *Vitacea polistiformis,* in concord grape vineyards. J. Invertebr. Pathol. 45: 147–151.

Shapiro-Ilan, D. I., and R. Gaugler. 2002. Production technology for entomopathogenic nematodes and their bacterial symbionts. J. Ind. Microbiol. Biotech. 28: 137–146.

Shapiro-Ilan, D. I., D. H. Gouge, and A. M. Koppenhöfer. 2002a. Factors affecting commercial success: case studies in cotton, turf and citrus, pp. 333–355. *In* R. Gaugler (ed.), Entomopathogenic nematology. CABI, New York.

Shapiro-Ilan D. I., R. Gaugler, W. L Tedders, I. Brown, and E. E. Lewis. 2002b. Optimization of inoculation for in vivo production of entomopathogenic nematodes. J. Nematol. 34: 343–350.

Shannag, H. K., S. E. Webb, and J. L. Capinera. 1994. Entomopathogenic nematode effect on pickleworm (Lepidoptera: Pyralidae) under laboratory and field conditions. J. Econ. Entomol. 87: 1205–1212.

Shields, E. J., A. Testa, J. M. Miller, and K. L. Flanders. 1999. Field efficacy and persistence of the entomopathogenic nematodes *Heterorhabditis bacteriophora* 'Oswego' and *H. bacteriophora* 'NC' on Alfalfa snout beetle larvae (Coleoptera: Curculionidae). Environ. Entomol. 28: 128–136.

Smith-Fiola, D. C., S. A. Gill, and R. G. Way. 1996. Evaluation of entomopathogenic nematodes as biological control against the banded ash clearwing borer. J. Environ. Hort. 14: 67–71.

Smits, P. H. 1996. Post-application persistence of entomopathogenic nematodes. Biocontrol Sci. Technol. 6: 379–387.

Snow, J. W., C. R. Gentry, and M. Novak. 1985. Behavior and control of the peachtree borer and lesser peachtree borer (Lepidoptera: Sesiidae) in peach orchards permeated with (Z,Z)-3,13-octadecadien-1-ol acetate. J. Econ. Entomol. 78: 190–196.

Steel, R.G.D., and J. H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach. 2nd ed. McGraw-Hill, New York.

Stuart, R. J., and R. Gaugler. 1996. Genetic adaptation and founder effect in laboratory populations of the entomopathogenic nematode *Steinernema glaseri.* Can. J. Zool. 74: 164–170.

Williams, R. N., D. S. Fickle, P. S. Grewal, and J. R. Meyer. 2002. Assessing the potential of entomopathogenic nematodes to control the grape root borer *Vitacea polistiformis* (Lepidoptera: Sesiidae) through laboratory and greenhouse bioassays. Biocontrol Sci. Technol. 12: 35–42.

Woodring J. L., and H. K. Kaya. 1988. Steinernematid and heterorhabditid nematodes: a handbook of biology and techniques. Arkansas Agricultural Experiment Station, Fayetteville, AR.

Yonce, C. E., and J. L. Taylor. 1992. Effects of abamectin on peachtree borers in the laboratory and in peach orchards (Lepidoptera: Sesiidae). J. Entomol. Sci. 27: 78–84.

Received for publication 25 November 2005; accepted 3 January 2006.